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Sheedfar, Fareeba; Di Biase, Stefano; Koonen, Debby; Vinciguerra, Manlio

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REVIEW

Liver diseases and aging: friends or foes?

Fareeba Sheedfar,[1]* Stefano Di Biase,[2]* Debby Koonen[1] and Manlio Vinciguerra[3,4,5]

[1]Molecular Genetics, University of Groningen, University Medical Center Groningen (UMCG), Groningen, The Netherlands
[2]Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA
[3]Division of Medicine, University College London (UCL) – Institute for Liver and Digestive Health, Royal Free Hospital, London, UK
[4]Euro-Mediterranean Institute for Science and Technology (IEMEST), Palermo, Italy
[5]Department of Medical Sciences, Division of Internal Medicine, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy

Summary

The liver is the only internal human organ capable of natural regeneration of lost tissue, as little as 25% of a liver can regenerate into a whole liver. The process of aging predisposes to hepatic functional and structural impairment and metabolic risk. Therefore, understanding how aging could affect the molecular pathology of liver diseases is particularly important, and few studies to date have tackled this complex process. The most common liver disease, affecting one-third of the overall population, is nonalcoholic fatty liver disease (NAFLD), characterized by an intrahepatic accumulation of lipids. NAFLD can evolve into nonalcoholic steatohepatitis (NASH) in the presence of oxidative stress and inflammation. NASH is a serious risk factor for disabling and deadly liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). Old age seems to favor NAFLD, NASH, and ultimately HCC, in agreement with the inflamm-aging theory, according to which aging accrues inflammation. However, the incidence of HCC drops significantly in the very elderly (individuals aged more than 70) and the relationship between the progression of NAFLD/NASH/HCC and very old age is obscure. In this review, we discuss the literature and we argue that there might be an age window in which the liver becomes resistant to the development of injury; this needs to be studied to understand fully the interaction between age and liver diseases from a therapeutic perspective.

Key words: aging; cytokines; demography; injury; insulin/IGF-1 signalling; reactive oxygen species; mouse models; NAFLD; NASH.

Aging and liver function

The aging of a vast majority of the population is a very recent phenomenon that has emerged as a direct consequence of the rise in human life expectancy (WHO, 2002). Longer life span, however, does not necessarily mean a prolonged healthspan, as indicated by the steep rise in the number of elderly individuals suffering from chronic diseases, including metabolic and cardiovascular disease. Although aging is not a disease, it is considered the leading risk factor for all chronic diseases, accounting for 60% of all deaths worldwide (WHO, 2009). Aging refers to a multidimensional process of organism decline. The liver is a vital organ with a wide range of functions, including detoxification, protein synthesis, and production of compounds necessary for digestion. Specific age-related hepatic changes have been reported, such as increased hepatocyte size, increase in the number of binucleated cells, and reduction in mitochondrial number (Premoli *et al.*, 2009; Gan *et al.*, 2011). These changes may significantly affect liver morphology, physiology, and oxidative capacity. Also, in the elderly population, there is a one-third loss of hepatic volume and perfusion between the ages of 30 and 100 years (Wynne *et al.*, 1989), a loss which has been reported to interfere with hepatic phase 1 drug pharmacokinetics (Marchesini *et al.*, 1988; Wynne *et al.*, 1989; Schmucker, 2005).

At the level of single-cell populations, a sharp decline in hepatic regeneration following hepatectomy or chemical injury has been observed during aging (Bucher *et al.*, 1964; Stocker & Heine, 1971; Popper, 1986; Jin *et al.*, 2009a). Indeed, in young rats, all hepatocytes enter the cell cycle after 70% liver resection, whereas only one-third do so in the aged liver (Stocker & Heine, 1971). The molecular basis for the loss of regenerative capacity of aged livers is just starting to be elucidated and may involve CCAAT/enhancer-binding protein (C/EBP) family members (α, β, γ), GSK3β, HDAC1, and SIRT1 epigenetic and signaling pathways (Gagliano *et al.*, 2007; Jin *et al.*, 2009a, 2011; Timchenko, 2009; Jiang *et al.*, 2013). Interestingly, transgenic mice having undergone replacement of C/EBPalpha with C/EBPbeta, generating beta/beta alleles, are long-lived (Chiu *et al.*, 2004), and targeting GSK3β, HDAC1, and SIRT1 enzymatic activities has been proposed to improve the course of various age-related diseases (Lucas *et al.*, 2001; Willis-Martinez *et al.*, 2010; Guarente, 2011).

Worth noting is that aging is associated with a physiological increase in lipid accumulation in nonadipose tissues, including the liver (Petersen *et al.*, 2003; Slawik & Vidal-Puig, 2006). As with other organs, accumulation of lipids in the liver may compromise their normal functionality by promoting organ-specific toxic reactions, also known as lipotoxicity (Slawik & Vidal-Puig, 2006). Aging markedly increases the prevalence of the metabolic syndrome in the human population (Ford *et al.*, 2002). Accumulating evidence also points toward an increased prevalence of nonalcoholic fatty liver disease (NAFLD) with older age in humans (Floreani, 2007). NAFLD includes a wide spectrum of liver pathologies ranging from benign hepatic lipid accumulation (steatosis) to advanced nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and ultimately hepatocellular cancer (HCC) (De Minicis *et al.*, 2013). Aging has also been reported to significantly enhance the progression to NASH and fibrosis, thus predisposing to increased mortality in elderly subjects with NAFLD (Regev & Schiff, 2001; Frith *et al.*, 2009). However, is the higher prevalence of NAFLD seen in the elderly population a result of physiological changes intrinsic to the aging process or may it reflect age-long compensation for lifestyle-associated factors, like obesity and

Correspondence
Manlio Vinciguerra, PhD, Division of Medicine, UCL Institute for Liver and Digestive Health, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK.
Tel.: +44 (0) 20 74332874; fax: +44 (0) 20 74332852;
e-mail: m.vinciguerra@ucl.ac.uk

*These authors contributed equally to this work.

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nutritional oversupply? Is aging then an actual risk factor for liver diseases, or a bystander? This review discusses the evidence both supporting and confuting the interrelationship between aging and NAFLD, NASH, and HCC. We will not cover here the role of superimposed challenges such as viral hepatitis or alcohol consumption during aging.

Aging and nonalcoholic fatty liver disease (NAFLD)

NAFLD is the most common chronic liver pathology in the United States, affecting almost 40 million obese adults or 15% of the population. It is also the 4th most common reason for liver transplants. About 30.1 million obese adults contract liver steatosis and 8.6 million develop NASH (Angulo, 2002). NAFLD is regarded as the hepatic manifestation of the metabolic syndrome, a cluster of abnormalities that includes insulin resistance and predisposes to type 2 diabetes and cardiovascular disease. Insulin resistance, whether acquired or genetically determined, is found in approximately 60% of all patients with NAFLD. Insulin resistance is believed to be the primary cause of NAFLD. In a hyperinsulinemic state, there is a failure to suppress the flux of free fatty acid derived from the expanding adipose tissue mass, resulting in enhanced fat uptake by the liver (Tilg & Moschen, 2008). In addition, insulin continues to activate lipogenesis, thereby accelerating fatty acid synthesis and triglyceride accumulation in the liver, further driving NAFLD (Brown & Goldstein, 2008). Although NAFLD is a strong and independent predictor for the development of type 2 diabetes, the link between insulin resistance and NAFLD has not always been demonstrated and there are a number of studies reporting dissociation of NAFLD from insulin resistance in genetic mouse models and in patients (Aparicio-Vergara *et al.*, 2013; Sun & Lazar, 2013).

NAFLD is a progressive disease characterized by hepatic fat accumulation (liver steatosis) in the early stages and hepatic inflammation driving the transition from benign steatosis toward more advanced NASH (Fig. 1). While the disease is reversible in its early stages, its treatment becomes more complicated in the advanced state. If not treated, NASH may progress to cirrhosis, which is an irreversible state of the disease characterized by scars on the liver tissue that can possibly lead to HCC (Podrini *et al.*, 2013) (Fig. 1). Although it is much debated whether insulin resistance is a cause of age-associated metabolic disturbances or rather a protective adaptive mechanism (Barzilai & Ferrucci, 2012), it is generally believed that age is a risk factor for increased hepatic steatosis. Fat may accumulate in the liver as a result of multiple abnormalities of hepatic lipid metabolism, although the mechanisms that underlie this age-related liver steatosis are yet to be clearly defined. These mechanisms may include enhanced fat uptake, increased *de novo* lipogenesis, decreased β-oxidation, and/or decreased synthesis/secretion of very low-density lipoproteins (Cohen *et al.*, 2011). Consistent with this, β-oxidation has been reported to decline in livers with old age, involving reduced hepatic expression of nuclear receptor peroxisome proliferator-activated receptor (Sanguino *et al.*, 2004). Other studies have highlighted an important role for augmented hepatic-adrenergic signaling in the induction of liver steatosis with aging (Katz *et al.*, 1993; Ghosh *et al.*, 2012), although the specific mechanisms underlying β-adrenergic-mediated lipid accumulation with age remain undefined (Ghosh *et al.*, 2012). However, Jin *et al.* recently provided a clear molecular mechanism for the age-associated development of steatosis by demonstrating that p300-dependent regulation of chromatin structure during aging causes the activation of 5 key genes that drive triglyceride synthesis in the liver. Moreover, it was found that the p300-p-C/E

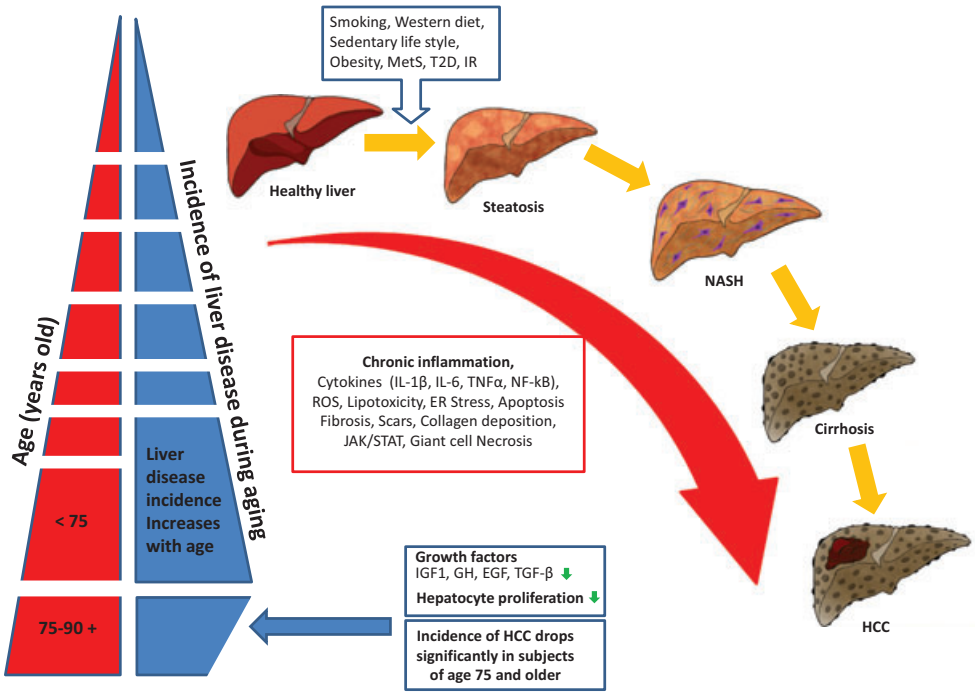

Fig. 1 Schematic illustration of the pattern of liver disease progression during aging. The incidence of liver diseases in humans increases with age up to 75 years. Interestingly, subjects aged more than 75 have a significantly reduced incidence of hepatocellular cancer (HCC) (left). The different states of liver disease progression (NAFLD, nonalcoholic steatohepatitis (NASH), cirrhosis, and HCC) and the respective molecular changes are represented on the right. See text for details.

BP-DGAT2 pathway is activated in human NAFLD, indicating that p300 and C/EBP proteins are essential participants in hepatic steatosis with age in both mice and humans (Jin *et al.*, 2013). In addition, hypercholesterolemia (Bonomini *et al.*, 2013), accumulation of reactive oxygen species, DNA damage (Aravinthan *et al.*, 2013), telomere shortening (Tomas-Loba *et al.*, 2013), decrease in autophagy (Amir & Czaja, 2011), activation of nuclear factor-kappa B (NF-κB) signaling (the master regulator of inflammatory responses) (Franceschi *et al.*, 2000), and metabolic dysfunction (Rodriguez *et al.*, 2007) have also been suggested as contributing factors. Detrimental factors that synergistically exacerbate the clinical spectrum of NAFLD during aging are cigarette smoking and a sedentary lifestyle (Breitling *et al.*, 2009; Booth *et al.*, 2011). Remarkably, the above-mentioned physiological decline in liver blood flow associated with aging is not affected by smoking habits (Vestal & Wood, 1980). Recent data also demonstrate a strong correlation between hepatocyte senescence markers and NAFLD (Park *et al.*, 2010; Aravinthan *et al.*, 2013). Although aging has been reported to increase lipid accumulation in the liver (Petersen *et al.*, 2003; Honma *et al.*, 2011; Ghosh *et al.*, 2012), Fontana *et al.* recently showed a different view of the impact of aging on NAFLD development (Fontana *et al.*, 2013). By putting young (2 months old), middle-aged (8 months old), and aged (18 months old) mice on a high-fat regimen diet for 16 weeks, Fontana *et al.* demonstrated that aging *per se* does not affect the development of simple steatosis (Fontana *et al.*, 2013). However, aging had an important role in inducing liver damage, increased proinflammatory M1 macrophage polarization and therefore increased inflammatory response and development of NASH (Fontana *et al.*, 2013). A recent clinical study by Noureddin *et al.* enrolling 735 nonelderly (18–64 years old, average 47 ± 11) and 61 elderly patients (>65 years old, average 68 ± 3), both groups displaying NAFLD, showed that elderly patients had a higher prevalence of NASH and advanced fibrosis, as well as other features of severe liver disease (Noureddin *et al.*, 2013). Though, a much larger population-based study to describe the prevalence of NAFLD in the elderly (the Rotterdam study), enrolling 2811 individuals, showed that NAFLD was found in 35.8% of participants aged <70 years (n = 455), in 36.6% of participants aged 70–74 (n = 809) years, in 39.6% of participants aged 75–79 years (n = 787), in 32.1% of participants aged 80–84 years (n = 495), and in 21.1% of participants aged older than 85 years (n = 265): overall, a clear trend of decline in the incidence of NAFLD in the very elderly as age advances (Koehler *et al.*, 2012). Even if hepatic steatosis is considered intrinsic to disease development, advanced liver fibrosis in NASH is often accompanied by a reduction in steatosis to the point of complete fat loss (van der Poorten *et al.*, 2012). This paradoxical but well-replicated finding is known as burnt-out NASH and may be related to a number of proposed mechanisms including elevations in serum adiponectin levels (van der Poorten *et al.*, 2012), portosystemic shunting (Nosadini *et al.*, 1984), changes in mitochondrial metabolism (Caldwell & Crespo, 2004), the inflammatory, catabolic state accompanying cirrhosis (McCullough & Raguso, 1999), and collagen deposition in the liver (Noureddin *et al.*, 2013). Given that elderly patients have more severe liver disease as compared with nonelderly patients (Koehler *et al.*, 2012; Noureddin *et al.*, 2013), this resolution of hepatic steatosis may in part explain the reduction in NAFLD prevalence with advanced age. We propose that the discrepancy between the studies showing increased NAFLD prevalence and severity and those showing an attenuation is due to the fact that the former ones were conducted in middle-aged/old, rather than in very old mice and humans (Vinciguerra, 2012; Fontana *et al.*, 2013; Noureddin *et al.*, 2013).

Aging liver, caloric restriction, and growth hormones

Physical activity and nutritional habits are variables in lifestyle that decrease the incidence of some age-related diseases (Pekkanen *et al.*, 1987; Blair *et al.*, 1995), which have been positively associated with longevity. Also, in the last 80 years, caloric restriction (CR) has been demonstrated to increase the maximum life span in yeast, worms, flies, and rodents (Partridge, 2012). As previous studies in centenarians have shown (Tietz *et al.*, 1992; Chan *et al.*, 1999), overall liver function under normal physiological conditions is not dramatically impaired with aging. Only when placed under stressful conditions, such as hepatectomy or acute liver injury, the liver may take a long time for regenerating. One of the features of the aging process, which directly or indirectly affects liver function, is the gradual decline of growth hormone (GH) and insulin-like growth factor 1 (IGF1), which undergo a 50% reduction between the ages of 20 and 75 (Floreani, 2007) (Fig. 1). Components of the IGF1 pathway such as PI3K/AKT and GSK3beta (Jin *et al.*, 2009b) have been demonstrated to control hepatocyte senescence by increasing the levels of cyclin D3 and by holding them in a G1 phase through a mechanism mediated by C/EBPα. Under normal conditions, the old liver is not able to regenerate properly and, when stressed, will respond differently as compared to younger tissue (Smith & Pereira-Smith, 1996; Gagliano *et al.*, 2007). However, uncontrolled activation of this regenerative process has been demonstrated to be crucial for the development of HCC in a C/EBPα-dependent manner (Wang *et al.*, 2004). The natural decline of the PI3K/AKT and GSK3 axis during aging can be seen as a positive feature whereby these hormonal changes may drive the cell to enter a protective mode against the development of HCC (Wang *et al.*, 2004) (Fig. 1). The decrease of GH obtained with CR extends life span in a variety of *in vivo* models and has shown significant benefits in recovery from liver steatosis in humans undergoing prolonged fasting or a diet based on 500 kcal day^{-1} consumption for periods of 2.5 and 5 months, respectively (Harrison & Day, 2007). The upregulation of GH/IGF1 in hepatocytes is thus an example of antagonistic pleiotropy whereby cellular proliferation allows for a better recovery from liver injury but may lead to HCC. In addition to the modification of the IGF1/AKT axis and C/EBPα, aging also causes an increase in the protein levels of histone deacetylase 1 (HDAC1) which, in a liver-specific transgenic mouse model, led to the development of steatosis at a young age (Timchenko, 2009). An increased protein level of HDAC1 is also involved in tumor development (Halkidou *et al.*, 2004).

Inflamm-aging and liver function

As originally proposed by Franceschi (Franceschi *et al.*, 2000), the aging process is driven by an unbalanced stimulation/response of the immune system, characterized by increased levels of inflammatory markers such as cytokines, chemokine, reactive oxygen species (ROS), as well as decreased levels of antioxidant enzymes such as superoxide dismutase (SOD) and phase 1 detoxifying enzymes (*inflamm-aging theory*). According to this theory, the persistence of inflammatory stimuli over time represents the biological background favoring susceptibility to age-related diseases/disabilities (Fig. 1). It is important to stress that the natural decrease in GH/IGF-1 levels observed in elderly individuals is a feature that, although beneficial, has to be coupled with practices aimed at reducing the increased levels of inflammation in order to lead to healthy aging. Another physiological change that negatively influences liver function is the redistribution of adipose tissue from subcutaneous to visceral sites (Tchkonia *et al.*, 2010). In addition, in old age, fat is redistributed outside fat depots resulting in ectopic lipid accumulation in nonadipose tissues

including heart, skeletal muscle, and liver, even in lean individuals (Slawik & Vidal-Puig, 2006; Tchkonia *et al.*, 2010). This is associated with increased mortality and risk of disorders such as hypertension, atherosclerosis, hyperlipidemia, insulin resistance, and diabetes, all of which increase the chances of developing NAFLD (Tran *et al.*, 2008). Counteracting inflamm-aging and adipose tissue redistribution in older people and others with a lifestyle based on regular physical activity and reduced caloric intake may be key factors for healthy aging.

Aging and hepatocellular carcinoma (HCC)

About 8–26% of individuals with NASH progress to cirrhosis, putting these patients at a high risk of developing hepatocellular carcinoma (HCC) (Podrini *et al.*, 2013). HCC is among the top most common causes of cancer-related mortality worldwide (Bosch *et al.*, 2004). There are three potentially curative modalities: percutaneous ablation, most commonly called radiofrequency ablation, surgical resection, and transplantation. In the majority of cases, HCC arises in the setting of cirrhosis, but hepatitis B and C are the most common underlying etiologies (Nordenstedt *et al.*, 2010; Venook *et al.*, 2010). However, HCC can also develop in a background of 'pure' steatohepatitis (Rappa *et al.*, 2013). HCC is very rare before age 40 as it increases progressively with older age and peaks in incidence around ages 70–75 (El-Serag, 2011). The aging liver in rodents has been shown to provide a pro-proliferative environment (Pasciu *et al.*, 2006). Strong epidemiological data suggest that in humans the incidence of HCC drops steadily and significantly in individuals older than 75, and up to 90+ (El-Serag, 2011, 2012) (Fig. 1). Concomitantly with the rising rates of HCC, there is a shift of incidence from typically elderly patients to relatively younger patients between ages 40 and 60 (El-Serag, 2011). This occurs despite the fact that the number of elderly subjects diagnosed with HCC is expected to increase in developed countries because of the rising incidence of NASH-related liver disorders such as cirrhosis (Bugianesi, 2007). Are then the very elderly protected against developing HCC? The mice studies discussed above (Fontana *et al.*, 2013) are in line with the inflamm-aging theory, according to which aging accrues liver inflammation and induces NASH, but show that aging *per se* does not affect steatosis. The oldest mice group studied was fed a high-fat diet starting at an age of 18 months and was analyzed 16 weeks later at 22 months (Fontana *et al.*, 2013). A mouse of 18 weeks corresponds roughly to a human aged 56, and a mouse of 22 months to a human aged 69 years (Flurkey *et al.*, 2007), which is not considered very elderly. As noted, NASH is a common precursor of HCC, and there are only inconclusive data available on the progression of NASH in the very elderly (Gan *et al.*, 2011). The relationship between very old age, NASH, and HCC is complex and far from understood.

Conclusions

Due to the fact that every lifestyle embarked on by each of us is made up of a number of variables affecting the aging process and having different grades of severity, when discussing age-related diseases such as NAFLD, NASH, and HCC, biological age, which reflects health status rather than chronological age, should be carefully considered. The epidemiological and laboratory data discussed here suggest that there might exist an age window in which the livers of very elderly 'survivors' (mice or humans) become resistant to the development of injury. On the other hand, it is possible that the observed lower prevalence of liver diseases in old age may be the result of a survival bias, which is not possible to determine in cross-sectional studies. We propose that these phenomena need to be studied further to understand fully the interaction between age and liver diseases from a therapeutic perspective, and extending this understanding to other organs may uncover a potential mechanism of longevity in a wider context.

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